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Research paper

DNA Profile of White Male Rats Spermatozoa after Treatment with Tannins Beluntas (*Pluchea indica*)

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Abstract

Traditional plants developed and used as anti-fertility are still considerably rare in the modern da One of the recognized anti-fertility plants vailable in the tropical country of Indonesia is is the leaves of Beluntas plant. In general, Beluntas dried leaves contain 1.885% tannin. Past research on beluntas tannin that has been carried out, the fraction of tannins can reduce the potential fertilization of rat spermatozoa, decreasing the number of spermatogenic cells, has no effect on the amino acid composition of semens. However, these studies did not evaluate the role of the tannin fraction in mitochondrial DNA profile spermatozoa. The aim of research to study the leaf tannins giving beluntas in mtDNA genetic profile of rats spermatozoa. This type of research is experimental approach. The research sample include 12 male rats weights about 250-300 mg, at the age 3 months old. The design research is also conducted on: the control rat group, without being treated. Group II is divided into five treatment, the group was given beluntas leaf tannin. Giving beluntas leaf tannins administered orally as 0.8 ml (an effective dose of tannins in inhibiting fertilization of previous studies) to the rats every other day for 98 days. Observations when giving treatment with an interval of 49 + 3 days, 49-day + 16, 49 + 26-day, 49-day + 36, 49 + 49 days (previous studies) on spermatozoa mtDNA profile. Spermatozoa were taken from the epididymis organ. Analysis of mtDNA genetic profiles using PCR. Analysis of the data used quantitative descriptive. The results showed that the spermatozoa DNA molecular weight in the range of 599-611 bp. Beluntas tannin giving treatment showed no effect on the molecular weight of DNA base pairs spermatozoa male rats.

Keywords: *DNA Profile, Spermatozoa, Tannin.*

1. Introduction

Anti-fertility man per oral from medicinal plants has never been done before (Joshi et al, 2011; Gupta et al, 2006). Therefore, the traditional anti-fertility need to be developed (Choudhany et.al). One of the most potential anti-fertility plants is Beluntas. The most active compound form beluntas leaf is tannins. It is influence the process of spermatogenesis, testosterone levels and the number of tillers female white rats. Tannins help to reduce the potential fertilization of spermatozoa of rats (Susetyarini, 2010a). Levels of tannin in the leaves of fresh beluntas 0.61% and 1.885% for dry beluntas leaves (Susetyarini, 2009). Tannin has the properties as phenol and astringent taste. Tannins found to inhibit protein synthesis (Robinson, 2003).

The effect on male reproductive would inhibit protein synthesis. It can affect the quality of spermatozoa that played a role in the process of fertilization. Inhibition of protein synthesis had no effect on the amino acid composition and content of spermatozoa (Susetyarini, 2015) so it does not directly result in spermatozoa motility. Motility is determined by genetic mtDNA that play a role in fertilization (Sanches, et.al, 2003). Mitochondria have their own genetic material (Susmiarsih, 2010).

The composition of amino acids in spermatozoa on the process of spermatogenesis has not changed but that changed the amino acid levels (Susetyarini, 2015). Furthermore, needs to be studied more about mtDNA genetic profile of spermatozoa after exposure beluntas leaf tannins due role in motility. In the long term after clinical trials, the results of this study are expected to be utilized further in helping the family planning program, namely as a male anti-fertility drugs.

Spermatozoa is produced from the process of spermatogenesis in the seminiferous tubules. The quality of spermatozoa has affect to the fertilization success (Leo et.al, 1972; Chan et.al, 2009; Rose et.al, 2013). Based on the results of previous studies, tannins can reduce the potential fertilization (Susetyarini, 2010a). Potential reduction of fertilization due to the quality of spermatozoa decreased. Fresh tannin of beluntas is expected to result into clotting/aglitinin semens and disturb the anti-aglitinin sources, so it can reduce the level of motility and vitality (Winarno, in Prafiadi and Susetyarini, 2011). One of anti-aglitinin source is a plasma epididymis (Harayama et al, in Prafiadi and Susetyarini, 2011). The epididymis is an organ that is used for the process of maturation of spermatozoa.

Tannin can interfere with metabolic processes of proteins found in plasma. Plasma protein formed on various kinds of amino acids including lysine, histidine, arginine, cysteic, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, trosine, phenylalanin, and tryptofan (Neumark et al, in Prafiadi and Susetyarini, 2011). The anti-aglitinin process contained in plasma is coagulated through the tannin in a way secrete proteins that contain various kind of amino acids to the epithelial cells and flowed into the cauda epididymis, so that the protein anti-aglutinin can not be bound in the acrosome of spermatozoa (Harayama et al, in

Prafiadi and Susetyarini, 2011) and epithelial cells secrete active epididymal fluid required for spermatozoa while in the epididymis (Okamura et al, in Prafiadi and Susetyarini, 2011) become inactive.

2. Methodology

The study design is experimental design was the posttest control group design, is presented in Figure 1.

2.1. Population and Sampling Technique

The population in this study is the strain wistar of adult age (3-4 months) is derived from inbreed were treated from birth until it was used as a sample. The samples used were 12 male rats, healthy, from the rat population reared from birth.

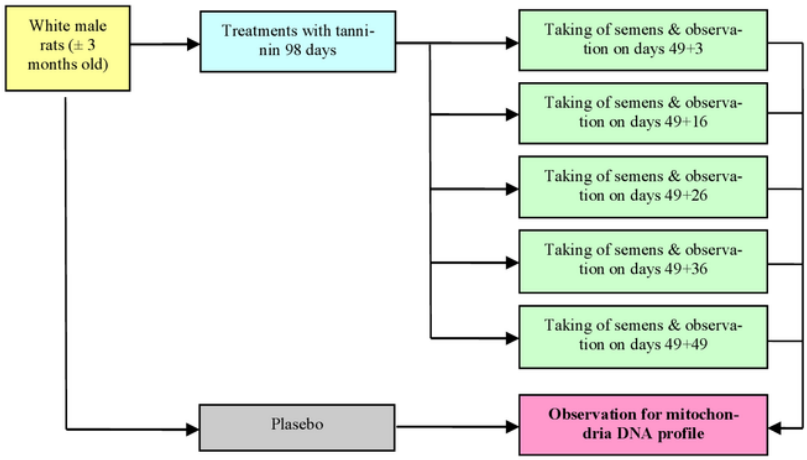

Fig. 1: Treatment scheme for tannin of beluntas leaves on male rats

2.2. Data Collecting Technique

The sampling technique including random sampling. The independent variable was the experimental animals treated beluntas leaf tannin and placebo (control). The dependent variables were: mtDNA profile of spermatozoa. Operational definitions of variables in this study are as follows: Treatment leaf tannin beluntas in this research, with a dose of 0.8 ml is given every other day for 98 days. Observations spermatozoa on day 49 + 3, days to 49 + 16, days to 49 + 26, days to 49 + 36 and 49 + 49 days to be taken of epididymal white male rats. Genetic profiles of mtDNA is helical covered, there are two strands, heavy (H) rich in guanine and light (L) which is rich in cytosine while heavy strands consist by 13 polypeptide (Wulandari, 2005). The research material is obtained from the leaves beluntas Materia Medica Batu. Beluntas leaves used is number 5 taken from the top branches.

Basically, this research is conducted in two main stages, the first one is isolation of tannin: 1) percirian bulbs by way WHO or Meteria Medica Indonesia, 2) phytochemical screening, 3) making extract: (a) maceration-percolation with ethanol; (b) soxlet insulation; (c) fractionated extracts; (d) the separation of components in fraction (Soediro, 2007) leaves beluntas be generated active substances in the form of tannin. Tannin extracts for using Lowenthal-Procter. Fractionation component of the active substance in the form of leaf tannin beluntas using fingerprints thin layer chromatogram (TLC) (LIPI in Susetyarini, 2009b).The second one is observations genetic profiles of mtDNA on 49 + 3 day, 49 + 16, 49 + 26, 49 + 36 and 49 + 49, during treatment to proving that tannin may affect mtDNA genetic profile during the process. Giving treatment performed daily at 0.8 ml. To ensure that all procedures are performed in this study are eligible to conduct, then before this study was undertaken, first research proposals submitted to the Ethics Committee (Animal Care and Use Committee) to obtain worthiness approval ratings and ethics.

When the sample were treated for 98 days, then performed the surgery for observation day 49 + 3. 49 + 16, 49 + 26, 49 + 36 and 49 + 49 at the time of treatment. Observations mtDNA genetic profiles when treated in accordance observations every 2 male white rats turned off by anaesthetized using isoflurane and decapitated (Belmonte, et al., 2000) and immediately the abdomen was opened to take the epididymis. Determination of mtDNA profile using PCR methods. Steps being taken: DNA isolation, separation of DNA, PCR process: the creation of a solution of silver staining, washing and observation phase, with a look at the mtDNA profile.

2.3. Data Collecting Technique

Data was analyzed by quantitative descriptive analysis.

3. Discussion and Conclusion

Data from the analysis of mtDNA profile spermatozoa male rats given beluntas tannin leaves, is presented in Figure 2 and Table 1.

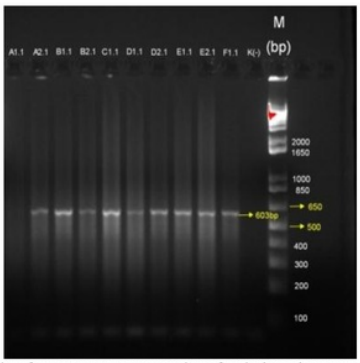

Fig. 2: DNA profile of spermatozoa rats males after being given tannin leaves beluntas

Figure 2 shows that the base pairs of DNA profiles after being tannin beluntas leaves on each treatment are presented in Table 1.

Table 1: The molecular weight of the DNA base pairs of male spermatozoa Rats after being Tannin leaves Beluntas

Treatment	Average Molecular Weight of Base Pairs (bp)
Tannin treatments of beluntas leaves 49+3 days	611.8
Tannin treatments of beluntas leaves 49+16 days	608.75
Tannin treatments of beluntas leaves 49+26 days	599.7
Tannin treatments of beluntas leaves 49+36 days	605.7
Tannin treatments of beluntas leaves 49+49 days	596.6
Control	599.6

The standard deviation of the molecular weight of 6,079 base pairs of DNA spermatozoa

Table 1 shows that the average molecular weight of DNA base pairs spermatozoa of 599-611 bp. According Nuraini, et.al (2012) states that the base pairs in white rats with a molecular weight of 603 bp with alkaline composition GAA TGG TGC GAT TTT GTC GTT GTT GGA T.

Figure 1 and Table 1 show that treatment of tannin for 49 + 16 day, 49 day + 26, 49 + 36 days, and 49 + 49 days showed a specific molecular weight to molecular weight of DNA primer 6,079 ± 603 bp. This situation occurred because the identified molecular weight were still within the range of molecular weight DNA primer. While the tannin treatment for 49 + 3 days showed a molecular weight of 611.8 bp and primer DNA with a molecular weight of 603 ± 6,079 primary means not specific to different DNA sequences. This is due to the inaccuracy and lack of proper optimization. In animals, where DNA damage can be experimentally induced in the paternal germ line, strong associations have been shown damage to the paternal genome and embryo development including effects on the new born and subsequent generations (Fernandez, et.al 2008; Delbies et.al, 2010)

These results indicated that tannin of beluntas do not changed mtDNA profile so that the average molecular weight of DNA base pairs is normal. Effect given by the tannin only on hormonal balances. Tannin including natural fitosteroid as an estrogen agonist by stimulating estrogen response causing hormonal disturbances. Characters fitosteroid chemical is similar to estrogen (steroid) hormone in the body. Presence of tannins resulting in damage to the structure of the plasma membrane of mitochondria of spermatozoa due to the process of free radical oxidation and lead to an increase in lipid peroxidation (Susetyarini, 2013).

Tannin work as well as steroid hormone directly in cells. These types of hormones capable of interacting with specific proteins in the cytoplasm and the so-called nuclear hormone receptors. The hormone receptor complex then interacts with DNA in a specific region and acts as a transcription factor then regulate the expression of genes through the process of protein synthesis. Scheme how the tannin is described in Figure 3 below.

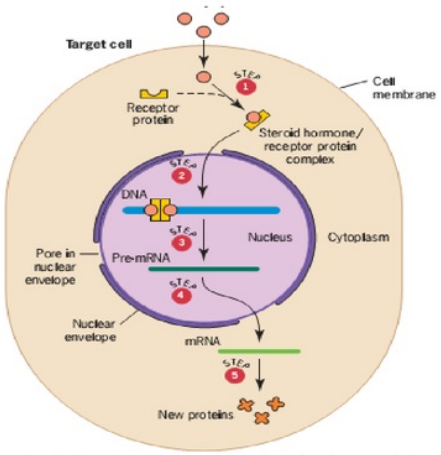

Fig.3: Mechanism of action of tannins in influencing the hormonal balance is based on the characteristics of chemical compounds in the form of fitosteroid (Snustad, 2012).

On the basis of these images is known that tannin does not change the mtDNA profile but controlling the hormonal effect. The existence of tannin damaging the structure of the mitochondria plasma membrane thereof free radical oxidation process and lead to an increase in lipid peroxidation. The formation of lipid peroxidation associated with increased formation of free radicals and correlated with decreased spermatozoa motility, decreased phosphorylation of proteins in aksonem and reduced ATP intracellular (Iwaki, et al., 1992; Asmarinah 2005, Susetyarini, 2013), as well as disrupt and hinder the process of spermatogenesis due the process of oxidation in the cell membrane of the testes.

This is in line with the treatment of the active compound tannin in lowering the percentage of motility, vitality and increasing the percentage of abnormal spermatozoa. The active compounds were administered single active compound is given in combination gives a different effect on the concentration of spermatozoa (Susetyarini, 2011b).

Beluntas leaf tannin compounds do not change but the mtDNA profile control hormonal control without causing changes or damage to mtDNA mice spermatozoa. Tannin compounds work as well as steroid hormones in cells, thereby being able to interact with specific proteins in the cytoplasm and the so-called nuclear hormone receptors. The hormone receptor complex then interacts with DNA in a specific region and acts as a transcription factor then regulate the expression of genes through the process of protein synthesis.

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